**Results:**
DNase I-sensitive probes were visualized within tissue biopsies of normal human bone marrow or Hodgkin lymphoma lymph nodes before therapy. Single cell analysis revealed all probe sites are confined to the euchromatin portion of the cell nucleus, and are visualized as ellipsoid clusters ranging in size from 25 - 100 nm (small), and 100 - 350 nm (large), to over 350 nm in overall diameter (giant). Within probed clusters, fine granulation is apparent of approximately 10 nm size, and the clusters are found near nuclear pores.
Normal bone marrow cells display a reduction in size and number of probed clusters during cell differentiation and maturation, while Hodgkin lymphoma apposed lymphocytes display an increase in size and number of probed giant clusters during in-vivo immune activation.
**Conclusions:**
Cluster of open regulatory elements (CORE) ultrastructural probes based on the DNase I-sensitivity of active transcription sites, can be useful in single-cell analysis within biopsied intact tissues.

**2439-Pos Board B209**
**Biophysical Characterization of a Recombinant Chromatin-Liposome Aggregates**
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Self-assembled aggregates of recombinant chromatin and liposomes were characterized by synchrotron small angle x-ray spectroscopy (SAXS) and optical microscopy. Recombinant nucleosome core particles (NCP) and 12-mer nucleosome arrays, where half of the negative DNA charge is neutralized by histone octamers, were used as a chromatin model. Liposomes of different charge and charge density were prepared from the either cationic DOTAP or anionic DOPG lipid molecules mixed with zwitterionic DOPC at different molar percentages.
Two distinct phases were identified in aggregates with low and high charge density cationic liposomes. The former appeared as translucent precipitate, probably due to higher water retention, compared to latter appearing compact and opaque, and exhibiting birefringence. Systematic SAXS measurements revealed chromatin-lipid bilayer complexes at 3-10% DOTAP, with lamellar distance around 160 Å for NCP, and 200 Å for arrays. At 10-100% DOTAP, proteins were displaced from DNA by cationic liposomes forming DNA lamellar phase. Confocal microscopy with triple fluorescent labeling displayed homogeneous distribution of DNA, proteins and lipids in both phases at the resolution scale determined by the diffraction limit.
Array adopted compacted 30-nm fiber and NCP formed columnar-hexagonal phase in the aggregates with anionic liposomes at 2-50 [$Mg^{2+}$] revealed by SAXS. These Mg-induced effects are known to occur in the absence of anionic liposomes. Variations of charge density and lipid concentration were found not to influence the dimensions of NCP columnar phase. Lipid molecules may prevent resolubilization of condensed chromatin by neutralizing excess of Mg ions.
Chromatin aggregates with liposomes of different charge may serve as a model of the eukaryotic nucleus where chromatin is surrounded by the nuclear envelope. The current results may demonstrate why cationic lipids destabilizing for chromatin are not prevalent in the nucleus, and how anionic membranes may influence the chromatin aggregation state.

**2440-Pos Board B210**
**Probing the Functional Integration of Mechanical Signals to Cell Nucleus**
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For cells to adapt to different tissues and changes in tissue mechanics, they must be able to respond to mechanical cues by changing gene expression patterns. These responses potentially involve major changes in nuclear organization and structure to reflect epigenetic changes in the nucleus. However, it is unclear how physical cues received at the plasma membrane integrate to the functional architecture of the cell nucleus. To probe this, we applied mechanical forces through magnetic particles adhered to the plasma membrane of single cells and mapped accompanying changes in cytoskeletal reorganization, soluble signalling intermediates, nuclear morphology and chromatin remodelling using high resolution fluorescence anisotropy imaging. Application of force on the plasma membrane resulted in spatio-temporal reorganization of actin cytoskeleton and chromatin assembly and the translocation of transcription co-factor Megakaryoblastic acute leukemia factor (MKL) from the cytoplasm to the nucleus. Taken together our results evidence a strong architectural coupling between physico-chemical networks and spatial organization of the chromosomes within the nucleus facilitating mechanotransduction.

**2441-Pos Board B211**
**Studying the Roles of Chromatin Insulators by Single-Molecule Methods**
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Eukaryotic chromosomes are condensed into several hierarchical levels of complexity: DNA is wrapped around core histones to form nucleosomes, nucleosomes form a higher-order structure called chromatin, and chromatin is subsequently organized by long-range contacts. The conformation of chromatin at these three levels greatly influences DNA transcription. One class of chromatin regulatory proteins called insulator factors set up boundaries between heterochromatin and euchromatin and generate long-range loops. In Drosophila, three types of insulators (Su(Hw), dCTCF and BEAF) have been shown to regulate transcription and organize chromatin at the higher level by the formation of long-range interactions that were proposed to be mediated by the coalescence of several insulator proteins into clusters (insulator bodies). In this project, we use Photoactivable Localization Microscopy (PALM) to quantify the number of insulator bodies per cell, to investigate their structure, composition and dynamics at super-resolution, and to correlate these results with genome-wide data.

**2442-Pos Board B212**
**Chromosome Organization by a Nucleoid-Associated Protein in Live Bacteria**
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We used super-resolution fluorescence microscopy and chromosome-conformation capture assay to study the distributions of major nucleoid-associated proteins in live Escherichia coli cells. HU, Fis, IHF, and StpA were largely scattered throughout the nucleoid. In contrast, H-NS, a global transcriptional silencer, formed two compact clusters per chromosome, driven by oligomerization of DNA-bound H-NS through interactions mediated by the amino-terminal domain of the protein. H-NS sequestered the regulated operons into these clusters and juxtaposed numerous DNA segments broadly distributed throughout the chromosome. Deleting H-NS led to substantial chromosome reorganization. These observations demonstrate that H-NS plays a key role in global chromosome organization in bacteria.

**2443-Pos Board B213**
**Mechanism and Function of Chromatin Positional Dynamics**
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Recent studies of the spatial organization of chromatin in interphase showed with 1Mb resolution that DNA is not as intertwined as expected but consists of knot-free globules [1]. Chromatin is territorialized with respect to the chromosomes, but also with respect to the expressed and silenced genomic regions. The globular organization facilitates the rapid folding and unfolding of local territories allowing a gene to be accessible for expression at any time [2].
In the present work we studied the dynamics of chromatin territories in interphase by measuring positional fluctuations of GFP-labeled histones H2B in mammalian cells (HeLa) using fluorescence intensity fluctuations spectroscopy. Spatio-temporal fluctuation maps of chromatin dynamics in single nuclei were determined by cross-correlation spectroscopy (STICS, [3]). We show that small territories move independently from each other confirming the blob-like structure observed in the static measurement [1].
In a second set of experiments, we studied the dynamics of chromatin on a single filament level by single particle tracking of GFP-tagged specific positions on chromosomal DNA (such as telomeres, centromeres). We hypothesize that chromatin is a kind of ‘‘active material’’ that combines passive (thermally driven) and active (motor-driven, ATP-dissipating) dynamics. We also hypothesize that chromatin is composed of blobs which move independently. The mean square displacement of locally labeled chromosomal DNA loci obeys a power law $\langle x^2 \rangle \sim t^{3/4}$ as expected for semiflexible macromolecules. A.Z. is Damon Runyon Fellow supported by Damon Runyon Cancer Research Foundation (DRG 2040-10).
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[3] B. Hebert et al., 2005, *Biophys. J.,* 88: 3601.